

## ***Ceriodaphnia dubia* 7-Day Chronic Toxicity Test**

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### **1.0 OBJECTIVE**

In laboratory tests designed to determine the toxicity of low salinity water samples, *Ceriodaphnia* neonates are exposed to test solutions for 7 days, after which the survival and reproduction is determined in each toxicant concentration. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as water quality parameters, such as dissolved oxygen, pH, conductivity, ammonia, alkalinity, and hardness.

In this procedure, water samples collected from field stations are divided into replicate containers in the laboratory. Single *Ceriodaphnia* neonates are placed into each replicate container and monitored for mortality and fecundity. After a 7-day exposure, daily survival and reproduction are used to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (US EPA 2002).

### **2.0 EQUIPMENT**

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

#### **2.1 Culture**

- Air stones and clean air system
- 2-liter beakers or similar volume containers
- 4:1 water prepared from Evian® and Nanopure water ( $25 \pm 1^\circ\text{C}$ .)
- YCT and *Selenastrum* for feeding, purchased from Aquatic Biosystems
- Disposable plastic pipettes

#### **2.2 Test Initiation**

- 30-mL disposable plastic cups (10 per sample concentration, leached with Nanopure water)
- 1000-mL volumetric flasks and pipettes for reference toxicant dilutions
- Water bath or environmental chamber
- Data sheets
- Gloves and appropriate safety gear (see MPSL lab safety manual)
- Sample vials for reference toxicant analysis (new polyethylene 60 mL)
- Plastic squirt bottles

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## **2.3 Water Quality**

- Meters and probes for measuring pH, dissolved oxygen, hardness, alkalinity, ammonia, and conductivity
- Thermometers (glass spirit thermometer and continuously recording thermometer)
- Graduated pipettes and hand pipette pump for water quality sampling
- Gloves and appropriate safety gear (see MPSL lab safety manual)

## **2.4 Dilution Water**

In every step of this procedure, use Granite Canyon Nanopure® water mixed with Evian® in a 4:1 ratio.

Conductivity should not exceed 2500µS at any time. Temperature for *Ceriodaphnia* tests should be  $25 \pm 1^{\circ}\text{C}$ .

Hardness (as  $\text{CaCO}_3$ ) should not exceed 700 mg/L.

## **3.0 EXPERIMENTAL DESIGN**

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, and comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of ten replicate test cups for each sample concentration. The quality of test animals and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites or receiving water is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and dissolved oxygen, pH, conductivity, and are measured at the beginning of the exposure. New and old dissolved oxygen and old pH are measured daily. Temperature is measured continuously, and hardness and alkalinity are measured at the beginning of the test.

## **4.0 PREPARATION OF SAMPLES FOR TESTING**

Because of the 48-hour holding time, tests will generally be initiated on the same day as sample receipt. Filter sample through a 25-µm screen and place appropriate sample volume in the constant temperature room. Allow oxygen concentrations to equilibrate below super-saturated levels. Aerate if super saturated.

## **5.0 CONTROLS**

### **5.1 Dilution Control**

The dilution control should consist of 4:1 culture water.

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### **5.2 Reference Toxicant Test**

A reference toxicant test must be conducted concurrently with every test to indicate the sensitivity of the organisms and the suitability of the test methodology. Reagent grade copper chloride ( $\text{CuCl}_2$ ) should be used as the reference toxicant for *Ceriodaphnia* tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 10,000  $\mu\text{g/L}$   $\text{CuCl}_2$  stock solution by adding 0.0268g reagent grade  $\text{CuCl}_2$  to one liter of distilled water in a volumetric flask. Cap tightly and mix thoroughly. Sample the reference toxicant stock solution at the beginning of the test for chemical verification of the copper concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N-reagent grade nitric acid.

Reference toxicant solutions should be ten replicates of 0, 10, 18, 32, 56, and 100  $\mu\text{g/L}$ . Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare concentrations according to dilution schedule. Start with the control solutions and progress to the highest concentration to minimize contamination.

All tests (sample and reference toxicant) must use neonates from the same culture. They must be handled in the same way and delivered to the test containers at the same time.

### **6.0 TEST INITIATION**

Measure temperature, dissolved oxygen, pH, conductivity, ammonia, alkalinity, and hardness. Each replicate cup receives 15 mL test solution. Load neonates from brood cups (from culture) using the blocking by known parentage method. Each cup contains a brood from a single female. These neonates are to be loaded into the same replicate of the control and samples. For instance, the contents of brood cup 1 should be loaded into the first replicates of the control and the samples. Load neonates into reference toxicant test in the same manner. Feed cups 200 $\mu\text{L}$  of *Selenastrum* and YCT (mixed in a 3:1 ratio).

### **7.0 DAILY TEST MONITORING AND RENEWAL**

#### **7.1 Survival, Brood Size and Transfer**

Live animals are transferred to new test solution daily. Survival and brood size are determined and recorded at the time of transfer. Aliquot new test solutions to a new set of 30-mL cups and place in the constant temperature room. Add 200 $\mu\text{L}$  of *Selenastrum* and YCT (mixed in a 3:1 ratio). Begin the renewal when the sample temperatures equilibrate. Set up the workstation for the renewal by placing a dissecting microscope on a light table. Place the old test board on the left of the scope and the new test board on the right. Remove a single cup from the old test board and its corresponding cup from the new board. Place the cups on the light table.

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Immobile *Ceriodaphnia* that do not respond to a stimulus are considered dead. The stimulus should be a gentle stream of water from a disposable transfer pipette. *Ceriodaphnia* that exhibit any response visible under the dissecting scope are considered living. Using the disposable transfer pipette, move the live adult *Ceriodaphnia* from the old cup to the new cup. If the animal has brooded record the number of neonates on the data sheet. If a dead brood is encountered, count the neonates to the best of your ability and place a letter "D" next to the number. If some portion of a brood is dead, note the number alive and dead with a slash between the numbers. When the adult dies and a brood remains, note the brood number, neonate number, and mark with an "X". Save water from the old cup for water quality measurements. Measure temperature, new dissolved oxygen and old dissolved oxygen and pH.

## **8.0 TERMINATING THE TOXICITY TEST**

After 8 days of exposure, or the sufficient production of control broods, final mortality and brood counts are made. Measure temperature, old dissolved oxygen, and old pH. Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

## **9.0 DATA HANDLING AND TEST ACCEPTABILITY**

Immediately after test termination, check the data sheet to determine whether dilution water and conductivity controls have acceptable survival. This toxicity test procedure is considered acceptable if *Ceriodaphnia* survival in controls is greater than or equal to 80%. Sixty percent of surviving females must have produced 3 broods, and surviving females must have produced an average of 15 neonates. Tests with temperature, conductivity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from  $25 \pm 1^{\circ}\text{C}$ ; acceptable dissolved oxygen concentration is 60-100% saturation.

## **10.0 REFERENCES**

U.S. Environmental Protection Agency. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. EPA-821-R-02-013. Office of Research and Development. Washington, DC.

## **11.0 TEST SUMMARY**

Species:	<i>Ceriodaphnia dubia</i>
Test Duration:	7 days
Renewals:	Daily
Organism Source	Culture

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Age of test organisms:	<24 hours
Conductivity:	<2500 $\mu$ S recommended
Dissolved oxygen:	4.0 - 9.67 mg/L recommended
Temperature:	25 $\pm$ 1°C recommended (range must not exceed 3°C required)
Light intensity:	Ambient laboratory illumination 10-20 $\mu$ E/ $\mu^2$ /s
Photoperiod:	16 hour Light: 8 hour Dark;
Test Containers:	30-mL plastic cups
Test solution volume:	15-mL minimum
Loading:	1 neonate per beaker
Feeding:	Daily – 200 $\mu$ L <i>Selenastrum</i> :YCT (3:1)
Water Quality:	DO, pH, conductivity, ammonia, alkalinity, hardness, and temperature
Reference Toxicant:	Copper Chloride (CuCl <sub>2</sub> )
Stock Solution:	0.0268 g in 1 liter of distilled water (10,000 mg/L).
Dilutions:	0, 10, 18, 32, 56, 100 $\mu$ g/L
Daily Monitoring:	Survival and Brood Size
Safety:	Wear protective clothing; read applicable MSDS, be familiar with the lab safety manual prior to testing.
Quality Control:	Fill out all data sheets completely. Be familiar with QA Project Plan prior to testing.
Acceptability Criteria:	Dilution Controls: $\geq$ 80% Surviving females: average 15 neonates Surviving organisms: 60% have 3 or more broods